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(71) Applicant *(for all designated States except US)*: **PHARMA MAR, S.A.** [ES/ES]; Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES).

(71) Applicant *(for SD only)*: **RUFFLES, Graham, Keith** [GB/GB]; 57-60 Lincoln's Inn Fields, London WC2A 3LS (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LOPEZ LAZARO, Luis** [ES/ES]; Pharma Mar, S.A., c/ Calera 3, Tres Cantos, E-28760 Madrid (ES). **FERNANDEZ SOUSA, José María** [ES/ES]; Pharma Mar, S.A., c/ Calera 3, Tres Cantos, E-28760 Madrid (ES). **ARMAND, Jean-Pierre** [FR/FR]; Institut Gustave-Roussy, 39-53, rue Camille Desmoulins, F-94805 Villejuif Cedex (FR). **RAYMOND, Eric** [FR/FR]; Institut Gustave-Roussy, 39-53, rue Camille Desmoulins, F-94805 Villejuif Cedex (FR).

(74) Agent: **RUFFLES, Graham, Keith**; Marks & Clerk, 57-60 Lincoln's Inn Fields, London WC2A 3LS (GB).

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(54) Title: TREATMENT OF CANCERS BY APLIDINE IN CONJUNCTION WITH A MYOPROTECTOR

(57) Abstract: Carnitine and other muscle protectors are useful to prevent side effects of aplidine and aplidine analogues.

TREATMENT OF CANCERS

The present invention relates to the treatment of cancers using aplidine or related compounds which are aplidine analogs.

BACKGROUND OF INVENTION

Cancer comprises a group of malignant neoplasms that can be divided into two categories, carcinoma, comprising a majority of the cases observed in the clinics, and other less frequent cancers, which include leukaemia, lymphoma, central nervous system tumours and sarcoma. Carcinomas have their origin in epithelial tissues while sarcomas develop from connective tissues and those structures that had their origin in mesoderm tissues. Sarcomas can affect, for instance, muscle or bone and occur in the bones, bladder, kidneys, liver, lung, parotid or spleen.

Cancer is invasive and tends to metastasise to new sites. It spreads directly into surrounding tissues and also may be disseminated through the lymphatic and circulatory systems. Many treatments are available for cancer, including surgery and radiation for localised disease, and drugs. However, the efficacy of available treatments on many cancer types is limited, and new, improved forms of treatment showing clinical benefit are needed. This is especially true for those patients presenting with advanced and/or metastatic disease. It is also true for patients relapsing with progressive disease after having been previously treated

Jimeno, J., "Exploitation of marine microorganisms and invertebrates: Anticancer drugs from marine origin", IBC Conf Discov Drugs from Nat Novel Approaches New Sources (Dec 8-9, London) 1994, 1994

Faircloth, G. et al., "Dehydrodidemnin B (DDM) a new marine derived anticancer agent (MDA) with activity against experimental tumour models", 9th NCI-EORTC Symp New Drugs Cancer Ther (March 12-15, Amsterdam) 1996, Abst 111

Sakai, R. et al., "Structure-activity relationships of the didemnins", Journal of Medicinal Chemistry 1996, 39 (14): 2819

Urdiales, J.L. et al., "Antiproliferative effect of dehydrodidemnin B (DDB), a depsipeptide isolated from Mediterranean tunicates", Cancer Letters 1996, 102(1-2): 31

Faircloth, G. et al., "Preclinical characterization of aplidine (APD), a new marine anticancer depsipeptide (MADEP)", Proc Amer Assoc Cancer Res 1997, 38: Abst 692

Depenbrock, H. et al., "In vitro activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells", British Journal of Cancer 1998, 78(6): 739

Faircloth, G. et al., "Aplidine (aplidine) is a novel marine-derived depsipeptide with in vivo antitumour activity", Proc Amer Assoc Cancer Res 1998, 39: Abst 1551

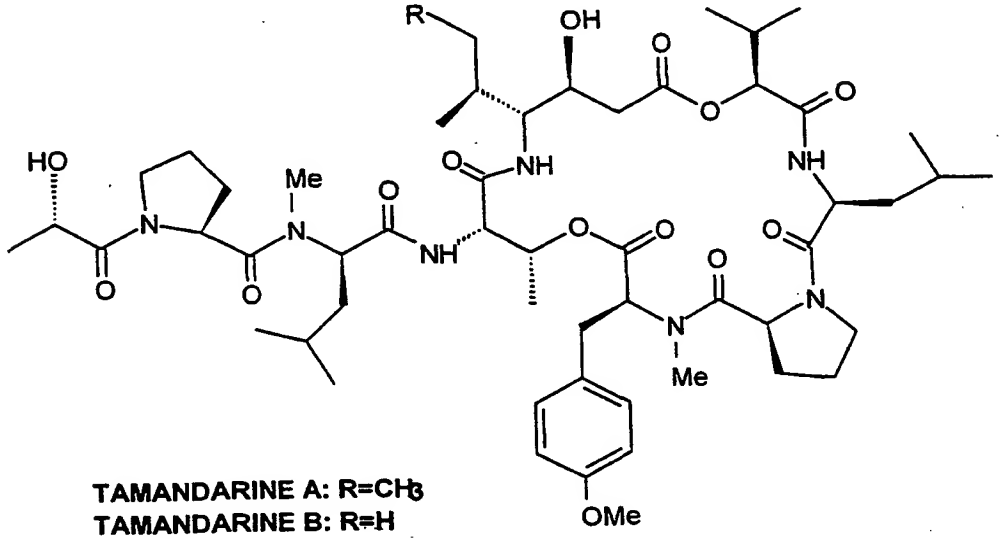
agent with activity against experimental tumour models. Annals of Oncology. 7 (Suppl. 1): 34, 1996).

Continuous exposure to low concentrations of aplidine inhibited the growth of a number of tumour cell lines, including non-Hodgkin's lymphoma, melanoma and breast, melanoma, ovarian and non-small cell lung cancers. The magnitude of effect was dependent on the time of exposure and appeared to be achievable at non-myelotoxic concentrations. Non-small cell lung cancer, breast cancer and melanoma cell lines were sensitive to a continuous exposure to aplidine at concentrations of >= 0.001 micromol/L. Aplidine had similar toxicity to doxorubicin against clonogenic haematopoietic stem cells (Depenbrock H, Peter R, *et al.* In vitro activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells. British Journal of Cancer. 78: 739-744, No. 6, Sep 1998).

Aplidine had significant activity against mice bearing human cancer xenografts. At a maximum tolerated dose of 2.1 mg/kg, aplidine produced near complete remissions in some animals with a treated/control (T/C) tumour ratio of 9%. At 1.25 mg/kg, significant activity was seen against gastric tumours (T/C 14%) and prostate tumour growth inhibition was also observed (T/C 25%) (Faircloth G, Grant W, *et al.* Preclinical development of aplidine, a novel marine-derived agent with potent antitumour activity. Annals of Oncology. 9 (Suppl. 2): 34, 1998).

Aplidine is related to other compounds of potential use against cancer, notably the didemnins. Aplidine is itself a dehydrodidemnin.

The article (d) relates to aplidine analogues called tamandarines, notably tamandarine A and tamandarine B:



TAMANDARINE A: R=CH3
TAMANDARINE B: R=H

Summary of Invention

We have developed improved methods to treat human patients with aplidine compounds, using muscle protectors such as L-carnitine. The aplidine compounds comprise aplidine itself, and aplidine analogues.

EMBODIMENTS OF THE INVENTION

The present invention provides a method of treating any mammal, notably a human, affected by cancer which comprises administering to the affected individual a therapeutically effective amount of an aplidine compound, being aplidine or an aplidine analogue, or a pharmaceutical composition thereof, and a skeletal muscle protector.

compound or in combination with any carrier or other pharmacologically active compounds.

Administration of an aplidine compound or the aplidine compositions of the present invention is based on a Dosing Protocol preferably by intravenous infusion. We prefer that infusion times of up to 72 hours are used, more preferably 1 to 24 hours, with about 1, about 3 or about 24 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be around 24 hours or even longer if required. Infusion may be carried out at suitable intervals with varying patterns, illustratively once a week, twice a week, or more frequently per week, repeated each week optionally with gaps of typically one week.

Examples of dosing regimes with and without carnitine are given in the following table:

Schedule	maximum tolerated dose, MTD	dose-limiting toxicity	recommended dose, RD
Aplidine weekly 24 hour infusion for 3 weeks, 1 week rest	4500	Muscular/ hepatic	3750
Aplidine weekly 1 hour infusion for 3 weeks, 1 week rest	3600	Muscular	3250
Aplidine 24 hour infusion every 2 weeks	6000	Muscular	5000
Aplidine 24 hour infusion every 2 weeks, L-carnitine daily	8000	Flu-like syndrome	7000
Aplidine 1-hour infusion for 5 consecutive days every 3 weeks	1500 x 5	Muscular/long lasting emesis	1350 x 5

a combination therapy. The other drugs may form part of the same composition as the aplidine, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

a) drugs with antimitotic effects, especially those which target cytoskeletal elements, including microtubule modulators such as taxane drugs (such as taxol, paclitaxel, taxotere, docetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);

b) antimetabolite drugs (such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues such as pentostatin, methotrexate);

c) alkylating agents or nitrogen mustards (such as nitrosoureas, cyclophosphamide or ifosphamide);

d) drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;

e) drugs with target topoisomerases such as etoposide;

f) hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuprorelin, goserelin, cyprotrone or octreotide;

g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;

h) alkylating drugs such as platinum drugs (cis-platin, carbonplatin, oxaliplatin, paraplidineatin) or nitrosoureas;

i) drugs potentially affecting metastasis of tumours such as matrix metalloproteinase inhibitors;

j) gene therapy and antisense agents;

k) antibody therapeutics;

l) other bioactive compounds of marine origin, notably kahalalide F or the ecteinascidins such as et-743;

changes among other mechanisms for signalling. A compound inhibiting VEGF action is expected to be inhibitory to such tumours.

Experimentally, aplidine has been found to have exceedingly high activity on human bladder cancer (giving complete remissions in some animal models), in accordance with the prediction.

Aplidine can be predicted to have a broad spectrum antitumour activity due to its effects on a large number of tumours.

The effect of VEGF is more relevant because it involves an inhibition of new blood vessels. In addition to effects on blood vessels, certain tumours required VEGF directly for cell growth (i.e. leukaemia, lymphomas, bladder tumours and ovarian tumours).

Responses in cancer patients have been observed in clinical trials with aplidine, demonstrating usefulness of the method of treatment.

Phase I clinical studies and pharmacokinetic analysis demonstrate that aplidine presents a positive therapeutic window with manageable toxicity in the range of dosage required for clinical efficacy in the treatment of cancer patients. In particular, the present invention is expected to be of benefit for treatment of renal cancer, melanoma, medullary thyroid carcinoma, lung neuroendocrine tumors, non-Hodgkin lymphoma, colorectal cancer, non-small cell lung cancer, among others.

The method consists of administration of drug by intravenous infusion over a period of 72 hrs or less at the recommended dose level (RD) with

The aplidine infusion solution should be administered intravenously, as soon as possible, within 48 hours after preparation. PVC and polyethylene infusion systems, as well as clear glass are preferred container and conduit materials.

The administration is performed in cycles, in the preferred application method, an intravenous infusion of aplidine is given to the patients the first week of each cycle, the patients are allowed to recover for the remainder of the cycle. The preferred duration of each cycle is of either 3 or 4 weeks; multiple cycles can be given as needed. The drug may also be administered each of the first days of each cycle. Dose delays and/or dose reductions and schedule adjustments are performed as needed depending on individual patient tolerance of treatments, in particular dose reductions are recommended for patients with higher than normal serum levels of liver transaminases or alkaline phosphatase, or bilrubin.

The Recommended Dose (RD) is the highest dose which can be safely administered to a patient producing tolerable, manageable and reversibly toxicity according to the Common Toxicity Criteria established by the National Cancer Institute, (USA) with no more than 2 out of 6 patients presenting any dose limiting toxicities (DLT). Guidelines for cancer therapy frequently call for administration of chemotherapeutic agents at the highest safe dose at which toxicity is manageable in order to achieve maximum efficacy (DeVita, V.T. Jr., Hellman, S. and Rosenberg, S.A., Cancer: Principles and Practice of Oncology, 3rd ed., 1989, Lipincott, Philadelphia).

measurable responses demonstrated evidence of clinical benefit to patients.

Definitions for patient toxicities are adopted from WHO Criteria and the responses determined following WHO Response Criteria.

Objective responses were obtained in patients with advanced and/or metastatic cancers refractory to previous treatments.

In particular treatment with this method has shown responses in cancer patients with advanced and/or metastatic disease, which exhibited progressive disease after having been previously treated with established therapies.

More generally, the invention involves the use of a muscle protector in conjunction with aplidine therapy. We have found in particular that carnitine is beneficial in treating myotoxicity that is associated with chemotherapy with the experimental drug aplidine. In Phase I trials when using a 24-hour infusion of aplidine, 4500 mcg/m^2 every week, then 6000 mcg/m^2 every second week, some subjects experienced some form of muscular and skeletal toxicity characterized by muscle cramping, pain and myopathic weakness. They also showed measurable increases in serum creatine kinase, an indicator of muscle breakdown and damage. When L-carnitine 4.5 g/day was added to the therapy (at doses of 1.5 g three times daily), or 0.1mg/kg (at doses of 0.033 mg 3 times daily) patients were able to tolerate doses of aplidine up to 6000 mcg/m 2 every second week. The beneficial effect, as seen in normal creatine kinase values, lasted throughout the study period of at least 13 weeks. Therefore, muscle protectors such as L-carnitine is

A phase I and pharmacokinetic study of aplidine, given as a 24h continuous infusion every other week in patients with solid tumours and non-Hodgkin lymphoma

Patient Characteristics		Tumour type	
Number of patients	43		
Median age, years (ranges)	52 (18-71)	Lung	6
ECOG performed status		Colorectal	8
0	19	Kidney	5
1	21	Breast	4
2	2	Pancreas	4
Prior radiotherapy	27	Lymphoma	3
Prior chemotherapy (No. regimens)		Ovary	2
1	7	Thyroid	3
2	5	Bone	1
≥3	29	Melanoma	1
		Prostate	1
		Uterus	1
		Mesothelioma	1
		Gastric	1
		Other	2

(G2 (G3) [G4]									
Transaminitis G2 (G3)	1	-	-	(1)	-	-	1	-	1
Hypertension G2	-	1	-	-	-	-	-	-	-
Neutrophenia G4	-	-	-	-	-	-	-	-	1
Pain central catheter G2	-	-	-	-	-	2	-	-	-

Characterisation of Muscular Toxicity (DLT)

Pat #27 - Male patient with medullary thyroid carcinoma treated at 6000 $\mu g/m^2$ weekly had symptomatic G3 CPK with G2 muscular pain. Toxicity recovered within 3 weeks after treatment discontinuation.

3 patients *(5000 and 6000 $\mu g/m^2$)* experienced a minor elevations of CPKs (≥G2), consisting of CPK MM (muscle) increase with no significant elevation of SPK MB (heart). A parallel elevation of the aldolase level was observed. Signs of improvement by using Carnitine supplements as skeletal muscle protectors are being reported. Muscle biopsies were performed in 2 patients; E/M: partial disappearance of thick filaments of myosin.

Pharmacokinetic Data

Aplidine appears to have a dose-linear PK profile (within the constraints imposed by the low sample size)

Relatively high plasma CL: median (quartiles) value 252 (192-415 $mL/min/m^2$)

High interpatient CL variability (coefficient of variation of CL 62%)

Intermediate to long t ½ with a median (quartiles) value of 23.8 (15.7-35.0 h)

Clinical features

The presentation is variable among patients. Mild cases have muscle cramps (at doses from 3200 mcg/m2 every 2 weeks), while iIn more severe cases the symptoms are associated to reversible increases in creatin-kinase (CK) reaching up to grade 3. In the dose-limiting cases there is weakness of proximal distribution. The effect has a delayed onset, appearing after 3 to 8 (median 4) infusions of the drug.

Pathological features

Light microscopy: just minimal necrosis or no changes at all (in most biopsied patients) or type II fiber atrophy (in a patient with gastric adenocarcinoma and concomitant long term 10 mg/d prednisone). Electron microscopy: aspecific accumulation of glycogen and autophagocytic vacuoles, being the most important change the disappearance of thick filaments.

Relationship to exposure

The maximum concentrations (Cmax) observed after 1 h infusion, before even hints of myotoxicity were found, were higher than those after doses related to myotoxicity 24 h infusion ruling out a Cmax relationship. The area under the curve (AUC) values in patients with myotoxicity tend to be high but not uniformly and not the maximum. Patients with dose-limiting myotoxicity had the longest terminal half-lives with the exception of a patient who received just 2 aplidine infusions, i.e. a treatment too short to be evaluable for myotoxicity. Another patient with myotoxicity had a short half life. Probably the relatively high AUC in some patients with muscular toxicity reflected the long half life. Hence, aplidine myotoxicity appears related to prolonged exposure rather than high exposure or concentrations.

systematic L-carnitine prophylaxis will be started and a new MTD and RD will be defined.

The possibility of tumor protection by L-carnitine was evaluated using a panel of 25 human tumor cell lines. Initial results are compatible with a lack of effect of L-carnitine on the antitumor activity of aplidine.

Figure 3 shows the evolution of acylcarnitines, where the thick lines represent 95% CI for the normal population for the respective parameter.

Acylcarnitine profile

It was measured by tandem mass spectrometry in plasma from a patient with clear toxicity (cramps + increased CK +weakness) at baseline, during toxicity and after aplidine treatment continued under L-carnitine protection. At baseline, there was decreased free L-carnitine, increased palmitoyl and steraroylcarnitine. During myotoxicity while on aplidine alone, free L-carnitine decreased while palmitoylcarnitine increased and stearoylcarnitine was stable L-Carnitine increased serum free carnitine up to supranormal values, while decreasing palmitoylcarnitine. Stearoylcarnitine was stable. Values for long chain acylcarnitines were less than half the diagnostic cutoff for CPT-II deficiency. Hence, this was not the underlying molecular defect (or at least not the only defect) present in this patient.

Conclusions

The dose-limiting factor for aplidine in 4 Phase I trials has been skeletal muscle toxicity. L-carnitine was assessed as a myoprotector for use in patients. The available clinical data demonstrates that L-carnitine prophylaxis enabled to increase aplidine MTD-RD by 33% and 40%

Claims

1. A method of treating cancer in a patient which comprises administering an aplidine compound in conjunction with a muscle protector.

2. A method according to claim 1, where the aplidine compound and muscle protector are adminstered as separate compositions with different dosing regimes.

3. A method according to claim 1 or 2, where the aplidine compound is aplidine.

4. A method according to claim 3, wherein the dosing of aplidine is in accordance with one of the following protocols:

24 hour infusion weekly for three weeks, followed by one week rest;

biweekly 24 hour infusion;

1 hour infusion weekly for three weeks every 4 weeks;

daily 1 hour IV infusion x 5 days q 3 weeks; and

3 hour infusion every other week.

5. A method according to any preceding claim, wherein the muscle protector is L-carnitine.

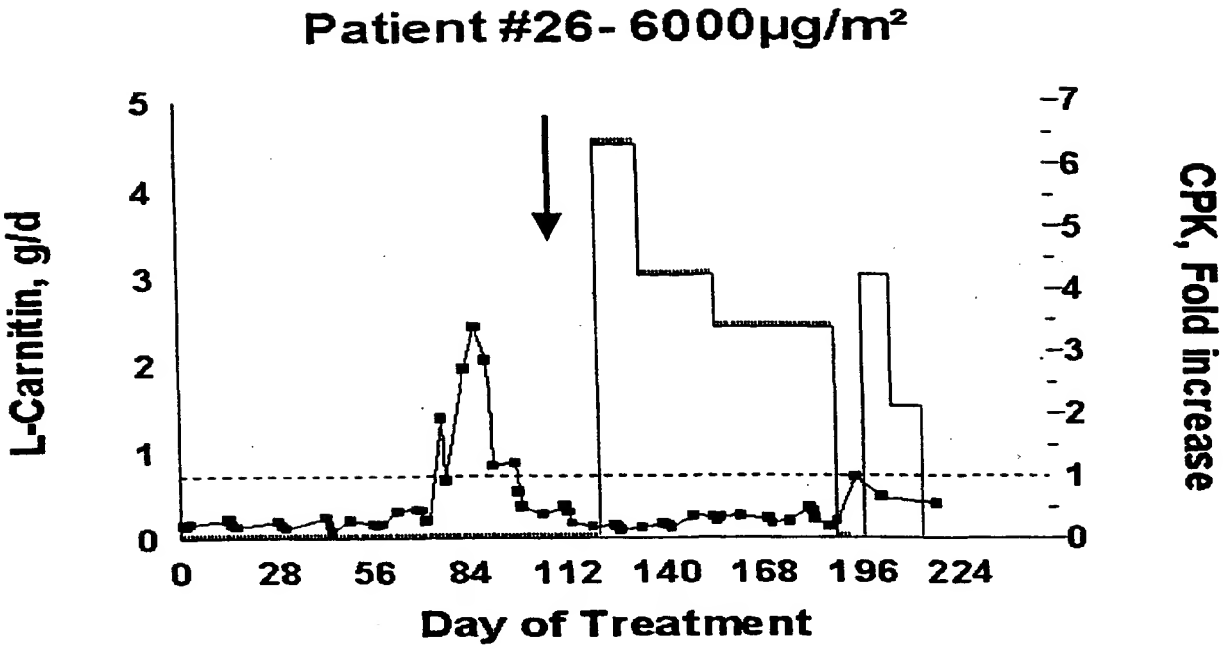

Patient #26- 6000µg/m²
L-Carnitin, g/d
CPK, Fold increase
0
1
2
3
4
5
0
1
2
3
4
5
6
7
0
28
56
84
112
140
168
196
224
Day of Treatment

Figure 1

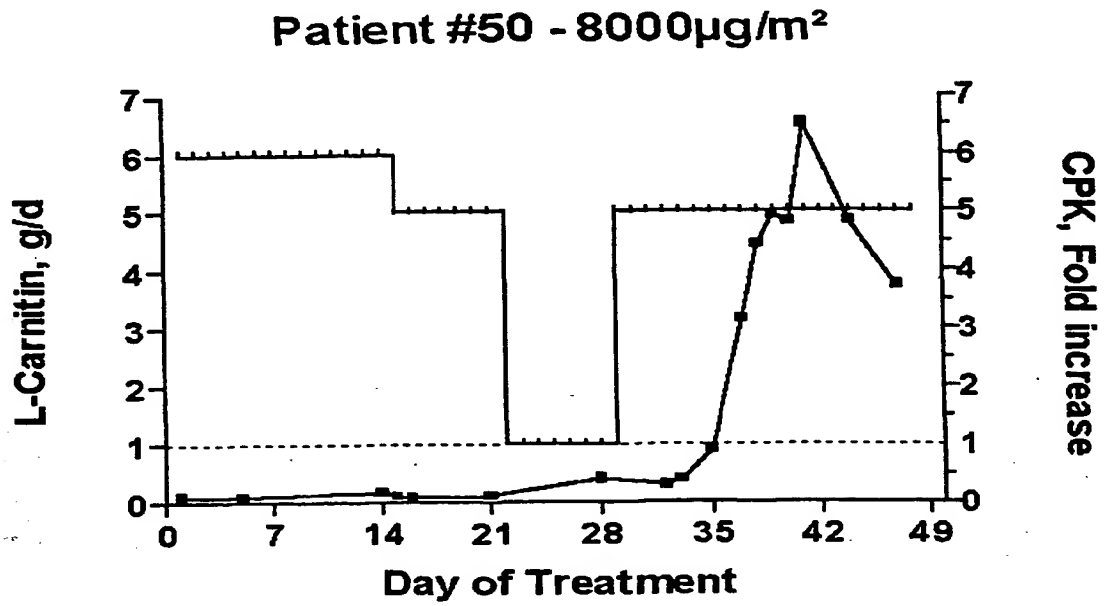

Patient #50 - 8000µg/m²
L-Carnitin, g/d
CPK, Fold increase
Day of Treatment
0
1
2
3
4
5
6
7
0
7
14
21
28
35
42
49

Figure 2

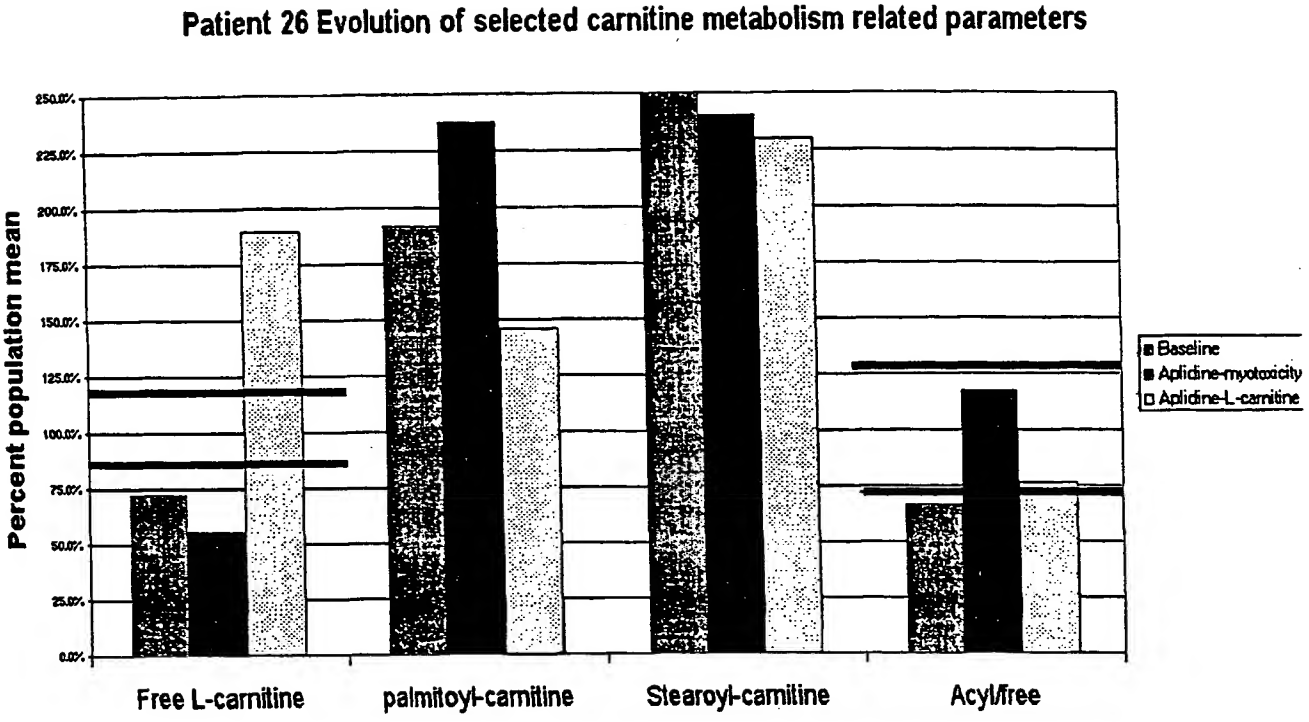

Patient 26 Evolution of selected carnitine metabolism related parameters
Percent population mean
250.0%
225.0%
200.0%
175.0%
150.0%
125.0%
100.0%
75.0%
50.0%
25.0%
0.0%
Free L-carnitine
palmitoyl-carnitine
Stearoyl-carnitine
Acyl/free
Baseline
Aplidine-myotoxicity
Aplidine-L-carnitine

Figure 3